providing a Ca2+ influx pathway (store operated calcium entry, SOCE) that is essential for proper immune cell function. Careful titration of the protein concentration of STIM1 and Orai1 thus determines the amount of Ca2+ influx and subsequently its downstream effects. Because changes in the degree and quality of glycosylation can affect immune cell development and function, we investigated the impact of altered glycosylation on SOCE. Indeed, inhibition of oligosaccharyltransferases increased SOCE in Jurkat T cells. To delineate the contribution of Orai1 and Stim1, we mutated their potential N-glycosylation sites. However, conventional replacement of the consensus asparagines by glutamines in STIM1 (N/Q) led to a reduction in Orai1 mediated currents. Other amino acid substitutions at the same positions led to variable degrees of current modification with one mutation leading to significantly increased current sizes. Interestingly, this mutation correlated with a change in Orai1 protein concentration and led to a change in the STIM1:Orai1 stoichiometry towards an optimized ratio for Orai1 activation. Noise analyses of Orai1 mediated sodium currents revealed an increase in the number of active channels, with little change in open probability or estimated single channel conductance. The phenotype of the mutant could only be partially mimicked by alteration of wildtype protein ratios between Orai1 and STIM1, suggesting an additional influence of the mutation on the EF-SAM domain stability and function. Our current data suggests that our gain-of-function STIM1 mutant may overcome the negative cooperativity which limits interaction of wildtype STIM1 with Orai1.

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Gating and Assembling Mechanisms of CRAC Channels

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Sustained calcium entry through Calcium Release-Activated Calcium (CRAC) channels is essential for T cell activation and proliferation via sensing the depletion of endoplasmic reticulum (ER) calcium-store by STIM1 subunits and the opening of pore-forming Orai1 subunits. However, the molecular mechanism underlying this process remains elusive. Using gain-of-function human Orai1 point mutants with Aspartate and Proline substitutions on a conserved Glycine residue (98) in the middle of transmembrane segment (TM) 1, we have previously suggested that the putative conformational change at this G98 site (gating-hinge) is a key step toward channel opening. Herein, we found in the mutation studies that the elongated side-chains at this 98-site could constitutively gate the corresponding channels through a conserved ''bent-hinge'' mechanism without affecting permeation pathway. We further demonstrated in Orai1-G98X mutants that the spontaneous opening of Orai1 channels is independent of STIM1 and store content. A truncated Orai1-G98X mutant without both intracellular N- and C- termini exhibited similar store-independent calcium entry as full-length Orai1-G98X mutants, indicating that Orai1 TM region is fully capable of and responsible for pore-forming and channel assembling. Moreover, truncated Orai3 proteins lacking both N- and C- termini remained responsive to 2-aminoethyl diphenylborinate stimulation, but not to store-depletion, indicating the structural requirement for channel assembling and pore formation. These results provide molecular details of the assembling and gating mechanisms of CRAC channels and support the Glycine ''gating-hinge'' hypothesis.

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Super-Resolution Imaging Reveals a Multi-Array Arrangement of Catsper Channel on the Sperm Tail

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Increase in intracellular Ca2+ initiates hyperactive motility of sperm, the high amplitude and often asymmetric movement of the tail. Cationic channel in sperm (CatSper) genes encode a complex of Ca2+ channel in sperm required for hyperactivation and male fertility. However, it is unclear how the CatSper-mediated Ca2+ entry increases flagellar bending and induces hyperactivated motility. Using high-resolution fluorescence microscopy based on high-accuracy localization of photoswitchable fluorophores, or stochastic reconstruction microscopy (STORM), combined with electron microscopy, we examined three-dimensional distribution of CatSper channels and flagellar proteins on the sperm tail. The CatSper channels form a unique three-dimensional multi-array arrangement, which may explain the characteristic patterns of hyperactivated motility.

3455-Pos Board B316

Contribution of the Epithelial Sodium Channel to Chondrocyte Regulatory Volume Increase

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Chondrocytes are the cells of articular cartilage, responsible for the production and maintenance of the extracellular milieu. They exist in a constantly changing osmotic environment and to survive such osmotic changes they must be able to quickly and effectively regulate their volume. Cells use regulatory volume increase (RVI) to oppose osmotic shrinkage. It has previously been shown that the epithelial sodium channel (ENaC) is important to this process in rat hepatocytes[1]. Here we investigate the possible contribution of ENaC to RVI mechanisms in canine chondrocytes.

Chondrocytes were isolated from cartilage according to standard methods[2]. Amiloride sensitive single-channel activity reversed at a membrane potential of -1 ± 5mV ($n = 5$), mean conductance was 9 ± 0.4pS ($n = 5$) and kinetics were slow. The calculated E_{Na} under these conditions was -6mV, which coupled to the very small conductance would be consistent with this channel being an ENaC. Channel open probability in control conditions was 0.3 ± 0.06 and decreased by $97 \pm 2\%$ after application of the ENaC inhibitor, amiloride (10μM; $n = 3$).

Upon exposure to hypertonic solution, cell volume decreased significantly by $35 \pm 3\%$ ($n = 5$; $p<0.001$). Within 20 minutes of reaching their smallest size, cells under control conditions had returned to $92 \pm 4\%$ of their original volume, not significantly different to starting volume ($p=0.07$). When 100nM benzamil, a specific ENaC inhibitor, was added to the hypertonic solution, cells shrank by $41 \pm 3\%$ ($n = 5$) and were unable to return to their original volume.

These data suggest that ENaC contributes to RVI in canine chondrocytes.

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Electrostatics in the NMDA Receptor Transduction Pathway Alter Gating

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Most excitatory synaptic transmission in the mammalian central nervous system is mediated by the neurotransmitter glutamate. Fast glutamatergic signaling is critical to cell-to-cell transmission, normal brain development, and learning and memory. NMDA receptors are a glutamate receptor subtype with high calcium permeability and slow gating kinetics. Although gating - the process of ligand binding/unbinding resulting in pore opening/closing - is a critical component of NMDA receptor function, our understanding of it remains incomplete. We used the GluA2 crystal structure to generate a rudimentary GluN1/GluN2A homology model. From this, we found that the NMDA receptor linkers, which connect the extracellular ligand binding domain (LBD) to the pore-forming transmembrane domain (TMD), contain a network of proximally lying charged residues. We hypothesized that the proximity of these charged residues might influence gating. To test this, we substituted proximal residue pairs with cysteines (to induce cross-linking). We found, for example, that currents in GluN1/GluN2A (E638C, K785C) receptors potentiated 150-fold when exposed to the reducing agent DTT. This suggests that these residues are proximal and have state-dependent positioning. To test that these residues interact electrostatically, we created charge reversal mutants and quantified single-channel activity. We found profound gating perturbations as each mutant showed significant differences in gating properties (open channel probability, mean closed time, and mean open time). We are currently in the process of performing double-mutant cycle analysis on double-charge reversal mutants to quantify the degree of charge-charge interaction. Thus, by making superficial, albeit critical, observations from our model, we are able to provide a potential physiological role for electrostatic interactions in NMDA receptor gating.

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Yellow Optogenetics with *Volvox* Channelrhodopsin Variants

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Channelrhodopsins (ChR) are light-gated ion channels and rhodopsins with internal ion conducting pores. In 2001 ChR1 and ChR2 cDNAs were identified in

Chlamydomonas reinhardtii data bases. The isomerization of the *all-trans*-retinal chromophore induces conformational changes of the protein that result in the opening of the channel pore to allow ion flow across the membrane. ChRs attract enormous attention because after expression in neuronal cells they can trigger action potentials upon blue light stimulation (450 nm). In 2007, we isolated two new ChRs from the fresh water algae *Volvox carterii* (VChR1 and VChR2). Notably VChR1 shows a red-shifted action spectrum peaking at 535 nm. Thus, VChR1 can be used to trigger action potentials in neurons by yellow light illumination [3]. Expression of ChR2 and VChR1 in different neuronal species enabled a distinct activation of the two cell types with blue and green light helping to understand neuronal circuits. But, a broader application of VChR1 was hampered because of its poor membrane localization and small currents in neurons. In this study we developed a well-expressing ChR with absorption characteristics similar to VChR1 by combining the N-terminal part of ChR1 and the C-terminal part of VChR1.

3458-Pos Board B319

APBSmem: A Tool for the Analysis of Membrane Protein Electrostatics

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Electrostatic forces orchestrate the folding of proteins, increase the binding of one protein to another and facilitate protein-DNA and protein-ligand binding. A popular means for computing the electrostatic properties of biological systems is to numerically solve the Poisson-Boltzmann (PB) equation, and there are several easy-to-use software packages available for carrying out these calculations on soluble proteins. We have developed a tool called APBSmem that performs these calculations in the presence of a membrane. Adaptive Poisson-Boltzmann Solver (APBS) is used as a back-end for solving the PB equation, and a graphical user interface (GUI) coordinates a set of routines that introduce the influence of the membrane, determine its placement and shape relative to the protein, and set the membrane potential. The software Jmol is embedded in the GUI to visualize the protein inserted in the membrane and the resulting electrostatic potential. We demonstrate the use of our software with three examples involving the calculation of the protein transfer free energy from water to membrane, solvation energy required to move an ion into a channel, and the gating charge of a molecular motion. We expect that the ease with which the GUI allows one to carry out these calculations will make this software a useful resource for experimenters and computational researchers alike. In particular, our built-in protocols should be appealing to researchers studying ion channel and transporter function.

3459-Pos Board B320

The Pivotal Twin-Histidine Element of the Escherichia Coli Ammonium Channel AmtB Functions as a Substrate Selectivity Filter

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Ammonium functions as both a primary nutrient and waste product and, thus, its transport across biological membranes is of fundamental importance. Because the uncharged form, NH_3, readily traverses phospholipid bilayers by simple diffusion the role of protein-catalyzed transport of the protonated species, NH_4^+, is unusually interesting. The Amt family of channels mediates the transport of NH_4^+ and is required for microbial growth when diffusion of NH_3 becomes limiting for nitrogen uptake. Whereas all other characterized channels facilitate downhill substrate movement, Amt proteins are active channels - hybrids between passive channels and active transporters - and concentrate NH_4^+ against a gradient. Amt family members function as homotrimers, with each monomeric unit carrying a pore for substrate conduction. Each pore is lined entirely with hydrophobic residues, save for a pair of conserved hydrogen-bonded histidines postulated to play a critical role in mediating NH_4^+ transport. We examined the impact that changes to this histidine pairing had on the function of one of the best-characterized members of the Amt family, the AmtB protein of *Escherichia coli*. Our initial analysis indicated that AmtB can accommodate, by either direct substitution or suppressor generation, acidic residues at one or both positions of the twin-histidine site while retaining good-to-excellent transport activity. Subsequent work shows that a number of mutant AmtB proteins carrying such alterations leak K^+ ions and that this leakage is energetically costly. These findings lead us to conclude that whereas the twin-histidine element is not required to conduct NH_4^+ it serves as a filter to prevent AmtB-mediated K^+ transport.

3460-Pos Board B321

Mapping Water Density to Design New Blockers Against a Viral Proton Channel

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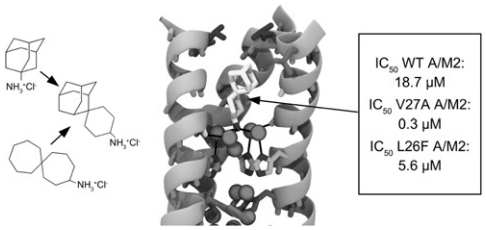
The influenza A virus utilizes a membrane-embedded proton channel, M2. Its functions are to acidify the viral interior and trigger the uncoating of its RNA, equilibrate the pH across the Golgi during replication, and allow a fully formed virus to bud from the host cell. One of the two classes of approved anti-influenza drugs contains amphiphilic molecules such as amantadine, that prevent extraviral H+ and water from accessing the pore of M2. Drug-resistant mutations (now pervasive through most of the flu strains) feature a more hydrated pore that destabilizes amantadine binding. We have used molecular dynamics simulations over extended times, reconstructed accurate water density maps, and identified several metastable positions of amantadine in the wild-type protein and its mutants. Using these data we designed new amine-based inhibitors, that fully suppress H+ conduction and viral replication in drug resistant strains.



3461-Pos Board B322

Effects of Substance P on Excitability of Dorsal Root Ganglion Neurons

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Some primary nociceptor neurons produce and release substance P (SP), a peptide neurotransmitter with well-described effects on second-order sensory neurons. However, the effects of SP on primary sensory neurons are less clear. We tested the hypothesis that SP acts on an autoreceptor by examining the pharmacological profile of rat DRG neurons sensitive to SP. Whole-cell patch clamp was used to measure the response of 89 cells to brief applications of pH 7 (27 positive cells), pH 6 (37 positive cells), capsaicin (22 positive cells), and ATP (15 P2X3 type positive cells). Sensitivity to SP was determined by an increase in cell excitability measured as the number of action potentials at the threshold and the slope of the stimulus-response curve (16 positive cells). There was also a decrease in excitability in 8 cells. Among the cells responding to SP by increasing excitability, the frequencies of sensitivity to pH 7 (67%) and pH 6 (88%) were higher than in non-responding cells (24% and 32 % respectively, $p<0.01$). The frequencies of sensitivity to capsaicin had a tendency to be higher in the SP responding cells (38% versus 22%, $p=0.33$). P2X3 type ATP currents were present in 15 of 73 (21%) SP non-responding cells, while none of the 16 SP positive cells presented this current ($p=0.1$). We conclude that the majority of SP sensitive neurons exhibit a pharmacological profile typical of nociceptors, although P2X3 currents were not present in these cells.

3462-Pos Board B323

Ion Channel Proteins that Spontaneously Insert into Lipid Bilayer Membranes: An Impedance Spectroscopy Study Employing Tethered Membranes

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The Chloride Intracellular Ion Channel (CLIC) protein family, the Annexins, the bacterial toxins Hemolysin (AH), Streptolysin, Perfringolysin Listeriolysin, Pneumonlysin, Ivanolysin and Colicins all possess unusual mechanisms for inserting into cellular and/or host membranes. They are representatives of proteins that spontaneously insert into membranes, by-passing the route for the incorporation of most integral membrane proteins. For example, the soluble form of the 240-amino acid polypeptide CLIC1 is known to exist in at least two conformations due to a large rearrangement of its amino terminus under the influence of oxidation. Oxidation promotes its binding to and insertion